The International Asian Research Journal 02(01): pp.43-50, 2014
ISSN: 2310-337X
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TEST OF ANTIMICROBIAL ACTIVITY OF TANNINS EXTRACT FROM GUAVA LEAVES TO PATHOGENS MICROBIAL

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Abstract: *This study aims to determine the antimicrobial activity of tannin extracts from guava leaves against pathogens microbial. The method used for qualitative analysis with the tannins are formed by the intensity of the color is blackish green FeCl3 compounds. In the quantitative analysis of tannins used variations of organic solvent (ethanol with a concentration of 30 % , 50 % , 70 %). Levels of tannins in the sample solution were calculated with Tannates Acid Equivalent (EAT). Test of antimicrobial activity using diffusion agar method. The results showed levels of tannins in leaves of guava with ethanol 30 %, the which is 2.351 mg /g , ethanol 50 % is 1.728 mg g , ethanol 70 % is 1.835 mg / g . The best solvent to extract the highest levels of tannins with ethanol 30 % by value of tannin levels 2.351mg /g. Tannin inhibitory activity on five different pathogens microbial. This is because the composition of the cell wall of the microbe fifth different. The results showed that the extracts rich in tannins can inhibit the growth of Escherichia coli, Pseudomonas aureginosa, Staphylococcus aureus, Aspergillus niger and Candida albican .*

Keywords: Tannin, Guava leaves, Pathogens microbial.

I. INTRODUCTION

Various types of plants contain natural preservatives or zar nature as antimicrobial and antioxidant. Antimicrobial compounds as biological compounds can inhibit the growth and have antimicrobial activity. Guava leaves (*Psidium guajava* L) is part of the guava tree commonly used as a traditional medicine to cure diarrhea and thrush. Guava leaves (*Psidium guajava* L) containing the active chemical compound saponins, flavonoids, tannins, eugenol and triterpenoids. Polyphenolic compounds dominate guava leaves are flavonoids (> 1.4 %) and tannins (BPOM,2004). Polyphenolic compounds are antibacterial compounds that can inhibit the growth of bacteria. According Fardiaz (1992), that may be antimicrobial compounds inhibit the growth of bacteria or mold (bacteriasatic or fungistatic) or be kill bacteria (bactericidal) or fungus (fungicidal) . Antibacterial compounds derived from plants could be phenolic substances such as flavonoids (Nakatani, 1988), organic acids (Yamanoto *et al*.,1984). Tannin compounds are polyphenolic compounds that are in plants, food and beverage (Makkar and Becker,1998) and water-soluble organic solvent (Haslam,1996). Tannins can be obtained from almost any kind of green plants, plants low level and high level it with the content and quality varies. Tannins are polyphenolic compounds that are very

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complex. The effectiveness of antibacterial compounds found in plant tannins eg guava leaves is influenced by the concentration of tannins. The higher levels of tannin antibacterial activity will increase. Preliminary studies to study the antibacterial activity of guava leaf has been implemented Faharani (2008), where the results showed that the water extract of guava leaves have antibacterial activity against *E. coli* at a concentration of 40 %, whereas in *S. aereus* extract showed no inhibitory activity, and the active compound is believed to have antibacterial activity of flavonoids, tannins and saponins. In guava leaves contain tannin by 9 %, which can be used as an antibacterial. Tannins can be used as an antibacterial because it has a phenol group, so that the tannins have properties like alcohol is an antiseptic that can be used as an antimicrobial component. Therefore this study is to assess the antimicrobial activity of tannin extracts from guava leaves against several pathogens microbial.

II. MATERIAL AND METHODS

Equipment and Materials

The tools used in this study include analytical scales, blenders, sieves, desiccator, petridish, stir bar, pipettes, Erlenmeyer flask, evaporator, filter paper, aluminum foil, vortex and UV-Vis spectrophotometer. Materials used in this study is the guava leaves, distilled water, technical ethanol 30%,50% and 70%. Folin Ciocalteu reagent, Na_2CO_3 7%, $FeCl_3$ 1%, nutrien agar and potato dextrose agar

Research Procedures

1. Sample Preparation of Guava Leaf Wind dried guava leaves for 1 week. Once dried blend to a powder and sieved using a sieve.
2. Guava Leaf Extraction. The sample has extraction by maceration. Weighed as much as 50 g of guava leaves, soaked in 150 mL of ethanol with a concentration of 30% ,50% and 70% for 24 hours and then filtered to obtain a filtrate. Treatment was for 3 days. Filtrate obtained together then evaporated to obtain ethanol extracts. The evaporated extract was cooled in a desiccator before further analysis.
3. Phytochemical test Tannins Using $FeCl_3$ 1% Phytochemical test is a qualitative test for the suspected presence of tannin in the extract of guava leaves. Phytochemical test conducted in this study that adding extracts with FeCl3 1% reagent indicated by the color change of green or blue-black ink. Phytochemical test using $FeCl_3$ 1% is used to determine whether a sample contains a phenol group is indicated by a green color blackish or dark blue after being added with $FeCl_3$ 1%, so if phytochemical with $FeCl_3$ 1% test gives a positive result it made possible the samples contained phenolic compounds and possible one of them is tannin. Because tannins are polyphenolic compounds. This was confirmed by Harbourne, (1987) classic way to detect simple phenol extract is added to a solution of $FeCl_3$ 1% in water, which cause the color green, red, purple, blue and black strong. Formation of green or blue-black ink on the extract after added with $FeCl_3$ 1% as tannins will form complexes with Fe^{3+} ions.
4. Determination of Levels of Total Tannins Manufacture of Standard Solution. Carefully weighed 10 mg gallic acid, then dissolved in distilled water and paid back the volume up to 10 ml to obtain a level of 1 mg / ml as a stock solution. And the stock solution pipetted their respective 10, 15, 20, 25 and 30 ml, was added to 0.2 ml of *Folin Ciocalteu* (after diluted with distilled water 1:1), homogeneously mixed for 10 seconds and then allowed to stand for 5 minutes. Then add 2 ml of Na_2CO_3 7% w/v (in distilled water), homogeneously mixed for 30 seconds, then paid back the volume to 5 ml with distilled water in a pint flask in order to obtain a final concentration of 2, 3, 4, 5, and 6 mg / ml. Allowed to stand for 95 minutes. Measured on a UV-Vis spektrofometer the maximum wavelength.

Preparation of Samples

Carefully weighed 0.1 mg of guava leaf extract was then added to 0.2 ml of *Folin Ciocalteu* (after diluted with distilled water 1:1), and then allowed to stand for 5 minutes. Then add 2 ml of Na_2CO_3 7% w/v (in distilled water), homogeneously mixed for 30 seconds, then paid back the volume with distilled water to 10 ml in a pint flask. From this stock solution pipetted 1 ml and diluted with water to 10 ml of distilled water. Allowed to stand for 95 minutes. Measured on a UV-Vis spektrofometer at maximum wavelength. Replication is done 3 times.

Antimicrobial Activity Test Diffusion Methods

Solid media that has been heated until melted, cooled to a temperature of ± 40°C, and poured in a sterile petri dish which was added 0.1 ml of a solution of active bacterial cultures, homogenized and allowed to solidify. Paper discs (diameter 6 mm) impregnated with a way to shed 20 ml. extracts from several concentrations (0, 1%, 1%, 10%, 20% and 30%) and negative control (DMSO) (Zakaria *et al.*, 2007). Subsequently incubated at 37°C for 18-24 hours for bacteria and fungi 35^{o}C for 48 hours. The diameter of the inhibition zone formed was measured using calipers (Volk and Wheeler, 1993).

Tannin Extract

Guava Leaf Extraction According Harbone (1987) to extract the tannins in a total network of plants required a solvent capable of dissolving polar compounds senywa especially tannins. Water is a good solvent for most of the tannins, but the best solvent is a mixture of organic solvents and water. Extraction is the process of separating a substance based on differences in solubility of the two immiscible liquids are different. Extraction method used in this study is extracted by maceration method. Maceration is a simple extraction method. Maceration is done by immersing the sample in an organic solvent. Organic solvents will penetrate the cell wall and into the cavity of the cell that contains the active substance so that the active substance will dissolve. Due to the difference between the solution concentration of active substance in the cell, then the solution is pushed out. The advantage this extraction method, is the method and the equipment used is simple and easily cultivated (Cheong *et. all*, 2005). Material to be macerated 50g soaked in a mixture of organic solvents (ethanol): water (1:3) with a concentration of 30%, 50% and 70% for 24 hours and treatment was repeated up to 3 times (Harborne 1987). Maceration is used because this process has a fairly high absorption effectiveness of the active substances contained in the leaves of guava include tannins. Fluid results maceration then evaporated with rotary evaporator to obtain a crude extract of guava leaves thick and brown, then to dry it using freezdryer. Concentration aims to determine the% yield as well as ease in terms of storage when compared in an extract that is still strong (there are still solvent). The yield difference on one of the six samples because the content of bioactive extracted by solvents, so that the results obtained yield was varied. Yield obtained can be seen in Table 1. Presentase yield calculated from the weight of dry extract extracted by looking at the weight of the initial sample.

Table 1. Sample Results Ekstrasktif Guava Leaf With Variations Solvent Concentration

Solvent	Dry Sample Weight (g)	Extract Weight (g)	Yield (%)
Ethanol 30%	50	4.593	9.186
Ethanol 50%	50	4.944	9.888
Ethanol 70%	50	5.686	11.371

Yield results obtained as shown in Table 1 indicate that the solvent composition of ethanol 70% has a higher yield is 11.37% rather than solvent composition.

Qualitative and Quantitative Test Tannins

Phytochemical test conducted in this study that adding crude extract of guava leaves with reagent $FeCl_3$ 1%. Results indicate a change in the color of blackish green, as shown in Figure 1.



Figure 1. Qualitative test Tannins Using $FeCl_3$

Determination of Content of Total Tannins

Tannins are generally defined as polyphenolic compounds have high molecular weight (over 1000) and can form a complex with the protein. Determination of total content of tannins in leaves of guava using total phenol method using *Folin-Ciocalteu* reagent and standard tanat acid. Determination of total phenol is used to determine the content of tannin contained in each sample. This

method has advantages including better color rendition, can minimize the differences at the time of testing and more specific (Rita, 2006). Folin method does not distinguish between types of phenolic components. The more the number of phenolic hydroxyl group, the greater the concentration of phenolic components were detected (Khadambi, 2007). Tabel 2 shows that the highest content of total tannins contained in the ethanol 30% tanin extract higher levels compared with the other extracts.

Table 2. Total Tannin Content Testing In Guava Leaf Mean Number of Deuteronomy solvent (mg/g)

Solvent	Replay			Number	Average (mg/g)
	1	2	3		
Ethanol 30%	2.118	2.484	2.452	7.054	2.351
Ethanol 50%	1.688	1.710	1.785	5.183	1.728
Ethanol 70%	1.871	1.796	1.839	5.506	1.835

Based on table 2 it can be seen that by using different solvents and concentrations, the amount of extractable tannins are also different, although the solvent used is the same.

Antimicrobial Activity Test Based Solvent Extracts Tannins Best Rich

The selection of the best solvent in this study is based on the total tannin assay with *Folin Ciocalteu* method. The best solvent to extract the highest levels of tannins with Ethanol 30 % by value of tannin levels 2.351mg/g . The selection was based on the best solvent extracts tannins that have the highest levels of value, so it can provide antimicrobial inhibition greater. This statement is confirmed by Zulaekah (2005) based on research results show that the higher the concentration or level of tannins in the tea leaf extract that is used in the manufacture of pickled eggs produce salted boiled eggs with the least number of total bacteria. This suggests that the higher levels of tannins in a sample it is possible to be more effective in killing or inhibiting the growth of bacteria. Antimicrobial activity assay in this study using a crude extract of tannin extract of guava leaves have not yet done but the process of separation or fractionation based phytochemical test and determination of tannins using the *Folin Ciocalteu* method was enough to show that in the extract contains tannin.

Testing antimicrobial activity of tannin-rich extract of guava leaves using *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *E. col*i as test bacteria, while *Aspergillus niger* and *Candida albicans* as test fungi and yeasts. The three types of these bacteria represent the type of Gram-negative bacteria and Gram-positive, in addition to play a role in food contamination and damage. Molds are also a lot of food contamination and damage. This is done to determine whether the tannin extract of guava leaves can inhibit the bacterial strains of Gram-positive and Gram-negative, fungi and yeasts, since there is the possibility of tannin which is a chemical that most of the spread in the plant is capable of inhibiting bacterial cell wall synthesis and damage the germ cell plasma membrane of Gram-positive and Gram-negative, so it is necessary to study the microbial activity of the tannin.

Extract concentration of ethanol 30% solvent used is 0, 1 %, 1 % 10%, 20% and 30% to determine the antimicrobial activity of tannin from guava leaves. Test the activity of tannin extract as antimicrobial done by measuring the inhibition zone formed around the discs. The test results demonstrated antimicrobial activity through inhibition diameter tannin extracts against microbes can be seen in Figure 3 below.

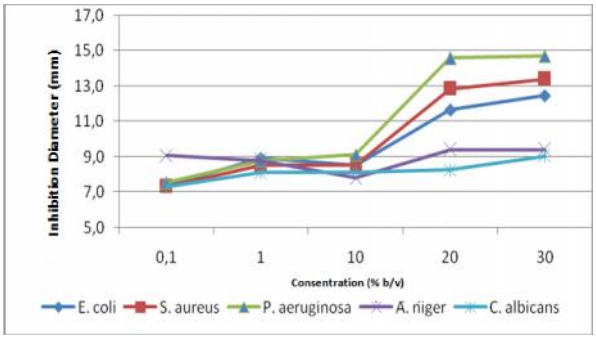


Figure 3. Inhibition diameter Tannins Extract Against Some Microbes

a. *E.coli*

ANOVA results showed that the treatment concentrations of tannin-rich extract of guava leaves provide a significant influence (P <0.01) against E. coli diameter inhibitors. Honestly significant difference test results (indicating that

the extract with a concentration of 0.1% was not significantly different from the concentration of 1% and 10%, but significantly different from the concentration of 20% and 30%. Figure 3 shows that with increasing concentration of the extract, the greater the diameter of inhibition which means that the greater the concentration of active ingredient that acts as an antibacterial, so its ability to inhibit the growth of *E. coli* bacteria growing.

b. *Staphylococcus aureus*

ANOVA results showed that the treatment concentrations of tannin-rich extract of guava leaves provide a significant influence (P <0.01) against *Staphylococcus aureus* bacteria inhibitor diameter. Honestly significant difference test results showed that the extract at a concentration of 0.1% was not significantly different from the concentration of 1% and 10%, but significantly different from the concentration of 20% and 30%. Figure 3 shows that with increasing concentration of the extract, the greater the diameter of inhibition which means greater levels of active ingredient that acts as an antibacterial, so its ability to inhibit the growth of *Staphylococcus aureus.*

c. *Pseudomonas aeruginosa*

ANOVA results showed that the treatment concentrations of tannin-rich extract of guava leaves provide a significant influence (P<0.01) against *Pseudomonas aeruginosa* diameter inhibitors. Honestly significant difference test results showed that the extract at a concentration of 0.1% was not significantly different from the concentration of 1%, 1% concentration was not significantly different from 10%, but the concentration of 0.1% was significantly different with concentration 10%, 20% and 30%. Figure 3 shows that with increasing concentration of the extract, the greater the diameter of inhibition which means greater levels of active ingredient that acts as an antibacterial, so its ability to inhibit the growth of *Pseudomonas aeruginosa*.

d. *Aspergillus niger*

ANOVA results showed that the treatment concentrations of tannin-rich extract of guava leaves significant effect (P <0.05) against *Aspergillus niger* diameter inhibitors. Honestly significant difference test results (Appendix 6b) showed that the extract at a concentration of 10% was not significantly different from the concentration of 0.1% and 1%, but significantly different from the concentration of 20% and 30%. Figure 3 shows that the extract concentration of 0.1% has been able to provide inhibition against *Apergilllus niger*, although with a low concentration of tannin extract has been functioning as antifungi

e. *Candida albicans*

ANOVA results showed that the treatment concentrations of tannin extract of guava leaves provide a significant influence (P< 0.01) against *Candida albicans* diameter inhibitors. Honestly significant difference test results showed that the extract at a concentration of 0, 1% was not significantly different from the concentration of 1 %, but significantly different from the concentration of 10 %, 20 % and 30 %. Figure 3 shows that with increasing concentration of the extract, the greater the diameter of inhibition which means greater levels of active ingredient that acts as an antifungi, so its ability to inhibit the growth of *Candida albicans*.

Tannin inhibitory activity on five different microbial above, this is because the five different microbes so that the composition of the cell wall is also not the same. The results of this study showed that the Gram-negative bacterium *E. coli* and *Pseudomonas aureginosa* with concentrations of low tannin -rich extract had inhibition zone greater value than the Gram-positive bacterium *S. aureus* at various concentrations. This can happen because the cell wall of Gram-negative bacteria are thinner than in Gram-positive bacteria that tannin is easier to attack the protein found in the cell wall of the bacterium *E. coli* and *Psudomonas aeroginosa*. Proteins in bacteria is one of the constituent components of the cell wall and plasma membrane , where the protein on the cell wall of the damaged or denatured bacterial cell wall will be easily penetrated by a chemical substance which causes impaired metabolism of bacteria. Cell wall of Gram-positive bacteria contain peptidoglycan mukopeptida or 90 % and the cell wall has a thickness of 25-30 nm , whereas the Gram-negative cell wall is thinner that has a thickness of 10-15 nm and consists of three layers . The inner layer is mukopeptida, the outer layer is composed of two layers, namely lipopolysaccharide and lipoproteins.

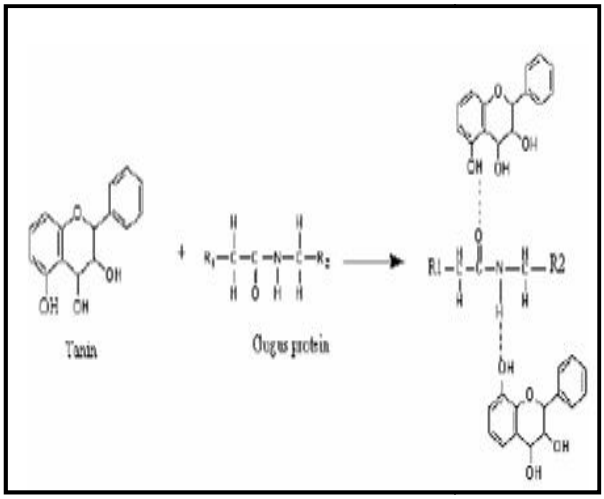


Figure 3. Tannins with Alleged Reaction between Protein Forces (Leemensand, 1991).

Tannins can form hydrogen bonds with the protein contained in bacterial cells, if the hydrogen bonds formed between tannins with proteins will be denatured proteins possibility that bacterial metabolism becomes impaired. The reaction of tannins with proteins to form a bond taninprotein. Section reactive protein and has the ability to bind with tannin is a peptide bond, hydroxyl and amide groups. Bonding is the dominant hydrogen bonding between the carboxyl groups of the peptide bond with the hydroxy groups of the tannins. The formation of hydrogen bonds between tannin with protein causes a conformational change in the protein molecule that biokimiawinya activity is reduced. This conformational change is called denaturation of proteins, clotting proteins normally preceded by denaturation that work well on isolistriknya point, so that the protein will undergo coagulation. Allegedly based on the reaction of tannins can inhibit the growth of bacteria *E. coli*, and *Psudomonas areuginosa*, *S. aureus*.

Tannin can also inhibit growth and kill bacteria by reacting with the cell membrane. Tannin compounds including polyphenols, these compounds can inhibit bacteria by destroying the bacterial plasma membrane is composed of 60% protein and 40% lipids are generally in the form of phospholipids, in the cell membrane of tannin will react with proteins to form hydrogen bonds so that the protein will be denatured, besides the tannins also can react with phospholipids found in cell membranes, resulting in tannin will damage the cell membrane, causing leakage of essential metabolites that inactivate the bacterial enzyme system. Damage to the cell membrane to prevent the entry of food ingredients or nutrients that are necessary for bacteria to generate energy will experience as a result of bacterial growth inhibition and even death (Volk and Wheller, 1988).

Inhibition of growth of *E. coli, S. aureus* and *Psudomonas areuginosa* by tannin extract of guava leaves suspected to be caused by this mechanism. Inhibitory activity of extracts rich in tannins can also serve as an antifungal indicated the presence of inhibition zones on *Apergillus niger* and *Candida albicans*. In general, it appears that the extract rich in tannins provide inhibitory effect on *A.niger* greater than *Candida albicans*. According Phongpaichit *et al*., (2004) provision of extractive substances to the fungus resulted in changes wrinkled and damage morphology of the fungus hyphae and makroconidia. This phenomenon can occur because of the leaking of the cell wall permeability or maybe some changes membram which resulted in loss of cytoplasma. The main component of the fungal cell wall structure is (1, 3) β- and 1, 6-β-glucan, chitin and manoprotein (Zacchino *et al*., 2003). Cells in the pack by the fungus cell wall carbohydrates that serve as a protective boundary cells that are essential for normal growth and development of fungi. (1,3)- β-glucan catalyzed by the enzyme sinthase (1,3) β-glucan .It is estimated that the compounds contained in an extract can inhibit the synthesis of polymers act by inhibiting cell wall enzyme sinthase (1,3) β-glucan (Zacchino *et al* .,2003). From these results it appears that the tannin extract was more effective in inhibiting the growth of the fungus *Candida albicans* and *A.niger*. Antimicrobial activity of extracts tannins inhibition as shown in Figure 4 .

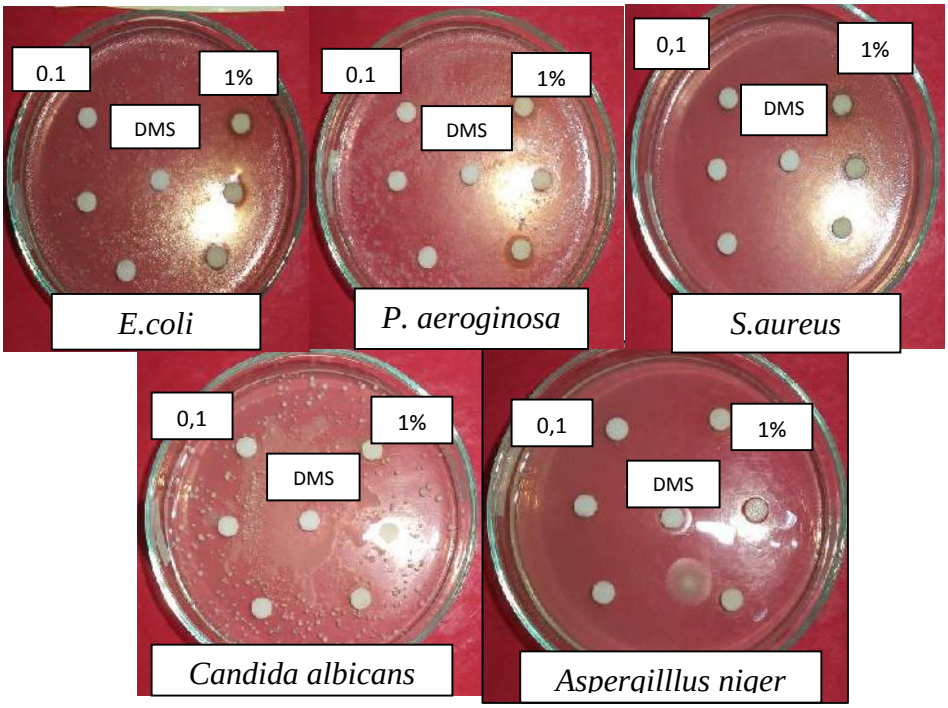


Figure 4 . Inhibition Activity of Antimicrobial Tannins Extract

III. CONCLUSION

1. The best solvent to obtain tannin extract of guava leaf is ethanol 30 %. This is supported by the calculation of tannin levels in each extract, tannin levels in the ethanol 30% extract of 2.351 mg/g.
2 . Tannin extract of guava leaves have antimicrobial activity against *E. coli, S. aureus , P.aureginosa , A.niger* and *C.albicans.*

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